

assemblies which alter the permeability of lipid membrane for ions and small molecules. These so called channel or pore forming proteins can either have one, two or three TM spanning domains. Albeit structural information is available for some of them, neither the number of monomers forming the functional pore or the proper orientation of most of the monomeric units within the assembly is available.

Computational methods are used to model the functional pore architecture of some of the proteins encoded by HIV-1, SARS-Co and Polio virus. Based on these findings novel pore motives need to be discussed and verified by experiments.

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Predicting Strained Regions for Folding and Protein-Protein Interaction in Bacterial Outer Membranes

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Beta-barrel membrane proteins are found in the outer membrane of Gram-negative bacteria, mitochondria, and chloroplasts. Exposed on the surface of bacterial cells, many beta-barrel proteins are initial sites of attachment by bacteriophages and bacterial toxins. Little information is known on the stability of local regions of these membrane proteins, and how they interact with each other. We have developed a model to quantify energy level of transmembrane strands in beta-barrel membrane proteins. We showed that our predictions on PagP are consistent with recent experimental NMR studies of protein dynamics [1] and mutant studies of folding stability [2]. We further found that the strands located in the interfaces of protein-protein interactions are considerably less stable by our model. We have further developed a method to predict interface of protein-protein interaction of beta-barrel membrane proteins. In a data set of 25 beta-barrel membrane protein structures with less than 32% pairwise sequence identity, we can predict whether the protein will form a monomeric or multimeric structure with 91% accuracy and identify the protein-protein interaction interface with an accuracy of 86% in leave one out tests using sequence information only. We also report results of predicting genome-wide protein-protein interaction in bacterial outer membranes.

[1] Evanics F., Hwang P.M., Cheng Y., Kay L.E. and Prosser R.S. (2006). Topology of Outer-Membrane Enzyme: Measuring Oxygen and Water Contacts in Solution NMR Studies of PagP. *J. Am. Chem. Soc.*, **128**, 8256-8264.
[2] Huysmans G.H.M., Radford S.E., Brockwell D.J. and Baldwin S.A. (2007). The N-terminal Helix is a Post-assembly Clamp in the Bacterial Outer Membrane Protein PagP. *J. Mol. Biol.*, **373**, 529-540.

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Molecular Dynamics Simulation of Transmembrane Helix Dissociation

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In a typical single-molecule force spectroscopy (SMFS) experiment with a transmembrane protein (TM), a protein surface loop or tail is grabbed by the atomic force microscope cantilever and helices unwind as the TM is pulled in a direction perpendicular to the plane of the membrane. This process is unrelated to the insertion of helical segments from the translocon into the bilayer in a direction parallel to the plane of the membrane. We now use molecular dynamics simulations (MD) to consider a SMFS experiment in which TMs are pulled apart in the plane of the lipid bilayer, so that the helix-helix interactions can be studied. Parallel-versus perpendicular-pulling energy. We calculated the work of separating the helices of the glycophorin A dimer in a sodium dodecyl sulfate (SDS) micelle. Pulling perpendicular to the membrane plane requires about four times the work as parallel pulling to separate the helices. Point pulling. Using MD, we pulled Ala 82 of glycophorin A in a direction parallel to the membrane plane. For 150 ps, the two helices separated smoothly, essentially as rigid rods. However, both Ile 76 side chains remain in van der Waals contact, locking the dimer together. Further pulling unfolds the backbone around Ala 82, until the Ile 76 groups separate after 600 ps. Similar results were observed for a two-helix fragment of bacteriorhodopsin in an SDS micelle. Pulling on Ile 52, the helices separate smoothly for 150 ps. However, during this motion, Leu 13 and Leu 61 remain locked in van der Waals contact. We conclude that SMFS of helical membrane proteins pulled in the direction of the membrane plane would provide valuable information about the interactions that stabilize integral membrane proteins. Experimental implementation will require development of tethering groups compatible with bicelles.

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The Interactions Of Secondary Structural Elements In The Achitecture Of Membrane Proteins

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Membrane proteins exist in an anisotropic environment which strongly influences their architecture, stability and folding. Previous studies have mainly focused on the transmembrane domains of alpha helical membrane proteins. However it is becoming increasingly apparent that their architecture is richer than a simple bundle of transmembrane alpha-helices, notably due to helix deformations and other membrane embedded structures. Here, we make use of available databases and tools to investigate the weight and role of structural heterogeneity in the supra molecular organization of membrane proteins. Using a non redundant subset of alpha helical membrane proteins, we have annotated and analysed the statistics of several types of structural element such as incomplete helices, intramembrane loop, helical extensions of helical transmembrane domains, extracellular loops and helices lying parallel to the membrane surface. The different structural elements and their composition were studied in relation to their immediate membrane environment. Calculation of hydrophobicity using different scales show that different structural elements appear to have affinities coherent with their position in the membrane. The considerable information content found in the amino-acid compositions of the different elements, suggests that the annotation scheme used might be useful for structural prediction. In a second step we have investigated the energetics of interaction between the structural elements. We show that while the folding in integral membrane polypeptides seems to be dominated by the interactions between transmembrane helices the same is not true of the assembly of complex membrane proteins. In most multisubunit membrane proteins the interactions between multiple domains are important in driving the assembly. Notably interactions at the surface of the membrane and between soluble domains are often very important.

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A Novel Pain Management Paradigm: Conformational Studies of the Mu-Opioid Receptor

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The mu opioid receptor (MOR) is a Class A G protein-coupled receptor. Naloxone, a MOR antagonist, has been shown experimentally to have partial agonist properties in a transmembrane helix 4 (TMH4) S196L/A MOR and full agonist activity in a triple mutant that combines the TMH4 mutation (S196L) with two mutations in TMH7, T327A and C330S (Claude-Geppert et. al, *J. Pharmacol. Exp. Ther.* 2005). We hypothesized that the loss of a serine in TMH4, the loss of a threonine in TMH7 and addition of a serine in TMH7 will modify the wild-type (WT) conformation of both helices and that this change may result in naloxone preferring the MOR activated state. The hydrogen bonding capacity of Ser/Thr residues in α -helices can be satisfied by an intrahelical hydrogen bond interaction, in either the *g*- or *g*+ conformation, between the O- γ atom and the *i*-3 or *i*-4 carbonyl oxygen. Ser/Thr residues in the *g*- conformation can induce a bend in an α -helix (Ballesteros et. al, *Biophysical J.* 2000), as well as changes in wobble angle and face shift (Zhang et al. *Mol. Pharmacol.* 2005). Using the Monte Carlo/simulated annealing technique, Conformational Memories, we explored the effect of Ser/Thr in *g*- on the conformation of WT MOR and the mutant TMH4 and TMH7s (Whitnell et. al, *J. Comput. Chem.* 2007). For TMH4, the (wobble angle, face shift) was found to be (-36.4°, 22.1°) for WT vs. (5.7°, 4.6°) for S196L, causing the top of TMH4 to move away from the TMH bundle. For TMH7, a significant difference was found in the average wobble angles of WT(52.52° \pm 21.72°) vs. mutant TMH7 (120.55° \pm 23.43°), causing the top of TMH7 to move into the bundle. [Support: NIH DA023905 and DA021358]

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The Kv1.2 Paddle Chimera Channel in a Lipid Bilayer

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The Kv1.2 paddle chimera structure (Long et al. 2007, *Nature* 450: 376) is the first voltage-dependent ion channel structure that reveals all residues at high resolution. In addition, fragments of lipid acyl chains and headgroups are also revealed. We present results of an atomistic molecular dynamics simulation of the Kv1.2 paddle-chimera tetramer in a POPC bilayer in excess water. The initial configuration includes the crystallographic waters and lipid fragments (reconstructed as POPC molecules) observed in the crystal structure (PDB ID 2R9R). Similar to previous simulation studies of Kv1.2 (Treptow and Tarek 2006, *Biophys. J.* 90:L64; Jogini and Roux 2007, *Biophys. J.* 93:3070), we find the basic side chains of the S4 helix in a polar environment formed by water molecules, lipid headgroups, and acidic side chains. As suggested by the crystal structure, the H-bond network that solvates each voltage sensor domain is interrupted by F233, a side chain highly conserved among Kv channels. The lipid fragments observed in the crystal structure are fully consistent with the lipid bilayer configuration and present a distinctive dynamics that contrasts with the bulk-like behavior of the lipid molecules far from the protein.